GENETIC ANALYSIS OF COTTAGE LAKE CREEK/BEAR CREEK AND ISSAQUAH CREEK NATURALLY SPAWNING FALL-RUN CHINOOK

ANNE R. MARSHALL
WASHINGTON DEPARTMENT OF FISH AND WILDLIFE
FISH PROGRAM, SCIENCE DIVISION
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Introduction

This report describes the genetic analysis of fall-run chinook salmon that were sampled on their spawning grounds in Cottage Lake and Bear Creeks (Sammamish River tributary system), and in Issaquah Creek during 1999. Natural spawners were sampled from Cottage Lake/Bear Creeks in 1998. The results of last year's genetic analysis provided evidence that Cottage Lake/Bear Creeks' chinook population could be considered a discrete unit from nearby Issaquah Hatchery. Differentiation from the Issaquah Hatchery population was low, but allele frequencies of the 1998 sample suggested that even if hatchery strays were a component of the natural spawner population, they were not necessarily dominant. Further evaluation of the genetic relationship of Cottage Lake/Bear Creeks population with Issaquah Hatchery was the objective for a second year of sampling.

Managers also wanted to genetically characterize natural spawners in Issaquah Creek. Given that most natural spawning occurs downstream of Issaquah Hatchery, and the presumed exchange of fish between hatchery and spawning grounds, genetic profiles of the two groups were assumed to be very close. For a broader perspective, relationships of the Sammamish area populations with hatchery and wild chinook populations in other Puget Sound locations would be evaluated similarly to last year's study.

The nature of genetic data used, allozyme gene allele frequencies, and the existing coast-wide standardized genetic data set were the same as described previously. If readers need a copy of last year's report, please contact the author (marsharm@dfw.wa.gov).

Methods

Tissue samples of muscle, eye, heart, and liver were taken from adult chinook post-spawning. Field samplers collected tissues from 100 chinook in Issaquah Creek, and from 107 in the Cottage Lake/Bear system, in which 23 were from Bear Creek and 84 from Cottage Lake Creek. Samples were kept on dry-ice and later stored at -75^{o} C. Scale samples were also taken and used for aging.

We used starch-gel electrophoretic methods to analyze genetic variation at 57 allozyme loci, and employed our standard baseline protocol. We screened for all known variant alleles in chinook salmon.

To ensure accuracy of interpretation and data entry, allozyme phenotypes were often resolved in two or more tissues, and/or with two different gel buffers. We used a WDFW computer program to check for discrepancies from all sources of phenotype scores. The final product of laboratory analysis was a multi-locus genotype for each chinook sampled.

Observed genotype frequencies at variable loci were compared to those expected in a population by Hardy-Weinberg (H-W) equilibrium tests. Significant deviations from expected genotype ratios can indicate a variety of conditions, for example, non-random sampling, gel scoring problems, or population mixture. Allele frequencies at all loci in each sample were calculated from genotype data. Allele frequencies provided the characterization of a population that was used for comparisons with other population samples.

I used a pair-wise *G*-test (log-likelihood ratio test) to test for homogeneity of allele frequencies between the 1998 and 1999 Cottage Lake/Bear creeks samples. It is expected that samples of the same population will differ little between years as long as the population is relatively large and experiences little emigration from others. I also used *G*-tests to compare allele frequencies among these two samples, the Issaquah Creek and Hatchery samples, and other Puget Sound population samples (Table 1) to see if significant differences (probability, $p,<0.05$) were detectable. One can expect allele frequencies to be divergent between two populations that are not exchanging genes at a high rate, and/or have different ancestries.

To further evaluate population relationships, I computed genetic distances (Cavalli-Sforza and Edwards chord distance) between all possible pairs of Sammamish area samples and the other Puget Sound samples. Distances were then used in cluster analyses (unweighted pair-group method), and in multi-dimensional scaling analyses to produce dendrograms and three-dimensional diagrams, respectively, for displays of relationships among samples.

Results

I found no overall significant deviations from H-W equilibrium among variable loci in either the 1999 Cottage Lake/Bear creeks sample (hereafter referred to as Cottage/Bear) or Issaquah Creek sample. Alleles observed at variable loci in both samples were those expected in Puget Sound chinook populations.

In the temporal comparison of the Cottage/Bear population, I did find significant differences ($p<0.005$) in allele frequencies between the 1999 and 1998 samples. Several loci showed relatively large differences, including sSOD-1. This locus had contributed substantially to differentiating Cottage/Bear from other populations in the 1998 sample analysis.

Comparing the Issaquah Creek and Issaquah Hatchery samples, I found significant differences ($p=0.01$) in allele frequencies, with one locus showing relatively large differentiation. Other pertinent *G*-test results for population sample comparisons were as follows:

No significant differences ($p>0.05$)

1999 Cottage/Bear Creeks versus 1992 Issaquah Hatchery
1998 Cottage/Bear Creeks versus 1999 Issaquah Creek

Significant differences with $0.05>p>0.01$

1999 Cottage/Bear Creeks versus 1999 Issaquah Creek
1998 Cottage/Bear Creeks versus 1992 Issaquah Hatchery

All *G*-test results of comparisons of the Cedar River population sample and the four Sammamish area samples were significantly different (p# 0.01)

The dendrogram resulting from a cluster analysis of genetic distances among the 1999 samples and other Puget Sound samples is shown in Figure 1. The 1999 Cottage/Bear sample appeared as a member of a grouping that included Green River Hatchery, Newaukum Creek, and Issaquah Hatchery samples. The 1998 Cottage/Bear sample joined first with the 1999 Issaquah Creek sample, and this pair appeared as somewhat of an outlier to the large grouping of south Puget Sound and Hood Canal samples. A multi-dimensional scaling diagram of genetic distance relationships among the same group of populations (Figure 2) showed a similar overall pattern of clustering, although 1999 Issaquah Creek sample appeared somewhat more closely aligned with 1999 Cottage/Bear, Green River Hatchery, and Issaquah Hatchery samples than with the 1998 Cottage/Bear sample.

In small chinook salmon populations, one can expect to find relatively larger variation in allele frequencies between years due to random processes (genetic drift) alone. Assuming the Cottage/Bear population is small enough to experience higher levels of drift, it would be appropriate to use allele frequencies from the combined annual samples to characterize the population. It would not be appropriate to pool samples if shifts in allele frequencies were due to a non-random event such as a large number of hatchery strays appearing in the population, or planting of non-local fish into the basin. I tried combining the two samples to further evaluate among-population relationships, although I do not assume to know the true dynamics the Cottage/Bear population.

Allele frequencies of the combined 1998 and 1999 Cottage/Bear samples were not significantly different ($p>0.10$) from those of the Issaquah Creek or Issaquah Hatchery samples. Genetic distances were calculated among the combined Cottage/Bear sample and the other population samples, and used in cluster analyses. The dendrogram in Figure 3 shows that the combined Cottage/Bear sample clustered similarly with the group of populations that clustered with the 1999 sample (Figure 1.) The Issaquah Creek sample clustered with this group, appearing as somewhat of an outlier. Using multi-dimensional scaling, the combined Cottage/Bear sample appeared closely aligned with other Sammamish and Green River basin samples (Figure 4).

The sex and age composition of the spawners comprising the 1999 and 1998 Cottage/Bear Creeks and 1999 Issaquah Creek genetic samples are shown in Tables 2a-2c. Aging by scale pattern analysis was

done by John Sneva, WDFW. The 1998 Cottage/Bear sample differed from the 1999 sample in that fewer females were included and three year olds were slightly more numerous than four year olds.

Interpretation and Discussion

The significant allele frequency differences found between the 1998 and 1999 samples of the Cottage Lake and Bear Creeks chinook population can indicate one or more circumstances. It is an expected result from temporal samples of a small population. Large annual fluctuations in abundance can also contribute to annual sample variability in small populations. However, small population size cannot be a final interpretation without information on extent of, and annual variation in, hatchery or non-local strays within the population. For example, an abundant return of hatchery spawners in one year might yield a proportionally larger number of strays into adjacent streams than usual. This could have a noticeable effect on a small natural population. It would be useful to know if there is any evidence that significantly more Issaquah Hatchery-origin fish occurred in Cottage Lake and Bear Creeks in 1999 than in 1998.

If genetic drift due to small population size was the major source of annual variation seen in Cottage/Bear chinook, then the combined years' sample likely represents an appropriate genetic characterization. If this is the case, the Cottage/Bear Creeks and Issaquah Hatchery populations have a similar allele frequency profile. Primarily, this indicates a common ancestral origin from chinook typical of south Puget Sound. It may also reflect some level of gene flow between them. Knowledge of the presence and magnitude of Issaquah Hatchery strays in Cottage Lake/Bear Creeks would allow a better assessment of gene flow. From the perspective of chinook salmon genetic diversity throughout the region, the Sammamish basin populations do appear closely related, and are more closely allied with populations in South Puget Sound than those from the Snohomish basin and northwards.

Similar allele frequency profiles do not rule out the possibility that Cottage Lake and Bear Creeks chinook are a relatively independent population from Issaquah Hatchery. The genetic data do not provide information about the influence of Issaquah Hatchery production on the persistence of the natural spawning population. An example of two independent populations with similar allele frequency profiles is Wenatchee River summer chinook and Hanford Reach (mainstem Columbia) fall chinook. These two populations are reproductively well-isolated by spawn timing and location, yet show very similar allozyme traits. Again, studying the pattern of Issaquah Hatchery straying will be instructive for determining independence of the Cottage/Bear population. It would also be useful to compare other biological traits of the two populations.

The differentiation found between Issaquah Creek and Issaquah Hatchery samples was relatively small. However, I would not expect to find significant differences in allele frequencies in Issaquah Creek natural spawners if they were largely a random group of Issaquah Hatchery-origin fish, as was expected. The Hatchery sample is not particularly old (1992) and only about two generations removed from the 1999 spawners. It would be useful to know if some event(s) has occurred in the hatchery, such as significant failure of a brood year, importation of gametes, etc. that might cause a shift in allele

frequencies. Perhaps the 1999 Issaquah Creek spawner sample is not a random subset of the Hatchery population, or they represent only a segment of the population, for example, fish with later return-timing, or some other trait that excludes them from brood stock collection. Information on hatchery-origin fish recoveries in Issaquah Creek would be useful for understanding the composition of natural spawners.

Acknowledgments

Bruce Baker, Cherril Bowman, and Norm Switzler, WDFW Genetics Laboratory, conducted and assisted with laboratory analyses. Kurt Fresh, WDFW, provided technical information and project guidance. Sampling in Cottage Lake and Bear Creeks was carried out by King County staff and Bill Mavros provided a data summary on the fish sampled. Samples from Issaquah Creek were provided by Muckleshoot Tribal staff.

Table 1. Other Puget Sound chinook population samples (n=15) used in this analysis.

Name	Yrs. of collection	Number sampled
Skokomish River fall-run	98, 99	229
Hamma Hamma River fall-run	99	55
Hoodsport Hatchery fall-run	88, 99	250
George Adams Hatchery fall-run	99	100
Nisqually River fall-run	98, 99	53
Clear Cr. (Nisqually) Hatchery fall-run	99	100
Kalama Hatchery (Nisqually) fall-run	99	100
Puyallup Hatchery fall-run	92, 93	150
South Prairie Creek fall-run	92, 93	86
Green River Hatchery fall-run	87, 88, 90, 98	399
Newaukum Creek fall-run	92, 93	144
Cedar River fall-run	93, 94	107
Issaquah Hatchery fall-run	92	99
Skykomish River summer-run	89, 93, 96	178
Snoqualmie River fall-run	88	101

Tables 2a-2c. Sex and age composition of chinook spawners sampled for genetic analysis in Cottage Lake and Bear Creeks and Issaquah Creek. n/a=not available.

2a. 1999 Cottage Lake and Bear Creeks						
Age/Brood Year						
Sex	2/97	3/96	4/95	5/94	n/a	Total
Male	1	21	29		9	60
Female		7	34		6	47
n/a						
Total	1	28	63		15	107

2b. 1998 Cottage Lake and Bear Creeks						
Age/Brood Year						
Sex	2/96	3/95	4/94	5/93	n/a	Total
Male	2	26	18		3	49
Female		8	11	1	0	20
n/a		1	1			2
Total	2	35	30	1	3	71

2c. 1999 Issaquah Creek						
Age/Brood Year						
Sex	2/97	3/96	4/95	5/94	n/a	Total
Male	3	16	19		10	48
Female		7	37	5	3	52
n/a						
Total	3	23	56	5	13	100

Figure 1. Dendrogram resulting from cluster analysis of pair-wise genetic distances among Sammamish Basin and other Puget Sound area chinook population samples. Populations are fall-run unless otherwise indicated. R.=River, H.=Hatchery, CR=Creek, SU=summer-run.

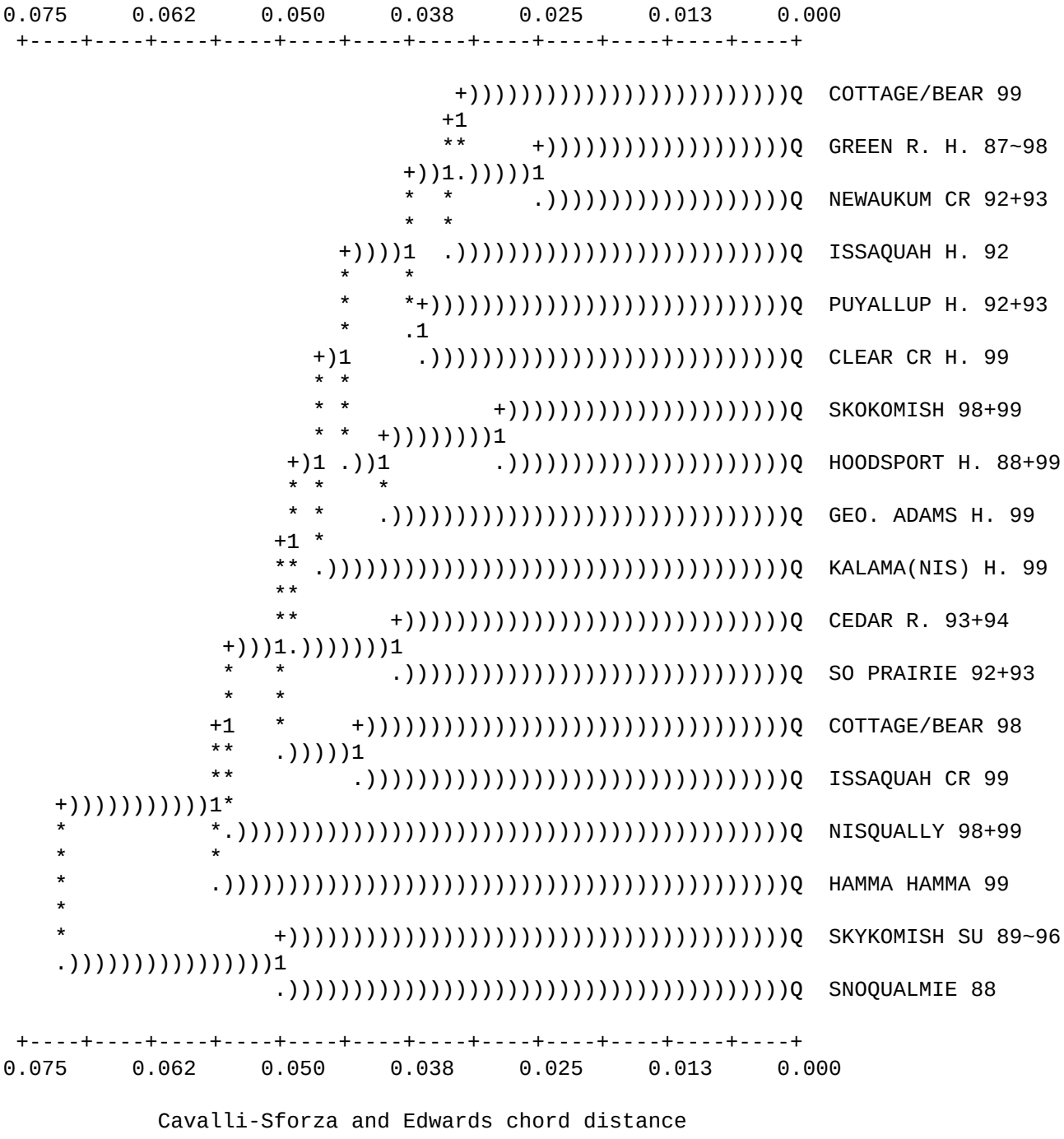

Figure 2. Multidimensional scaling diagram of genetic distances among each Cottage Lake/Bear creeks sample, other Lake Washington area, and south Puget Sound samples.

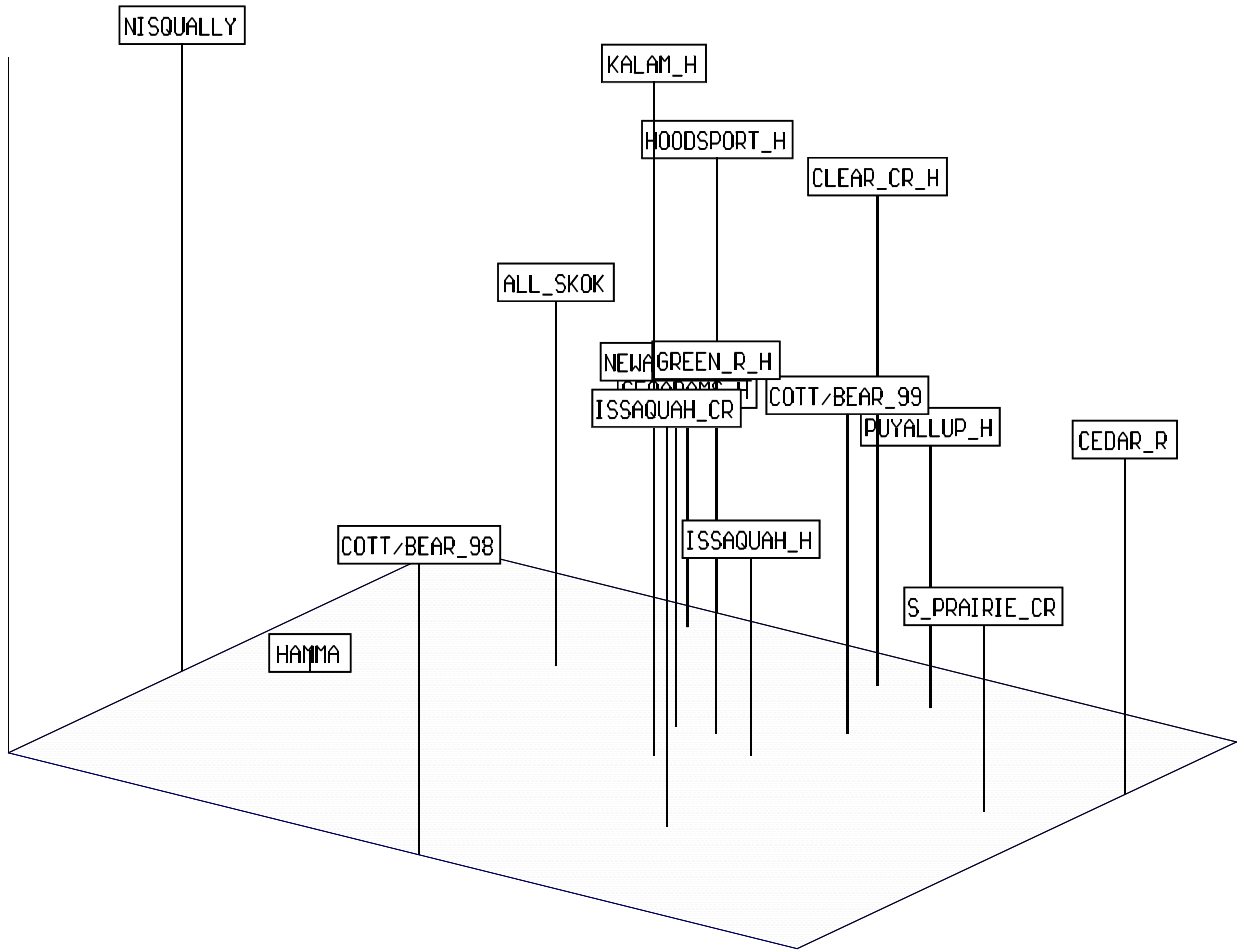

Figure 3. Dendrogram resulting from cluster analysis of pair-wise genetic distances among Sammamish Basin and other Puget Sound area chinook population samples. 1999 and 1998 Cottage Lake and Bear Creeks samples were combined. Populations are fall-run unless otherwise indicated. R.=River, H.=Hatchery, CR=Creek, SU=summer-run.

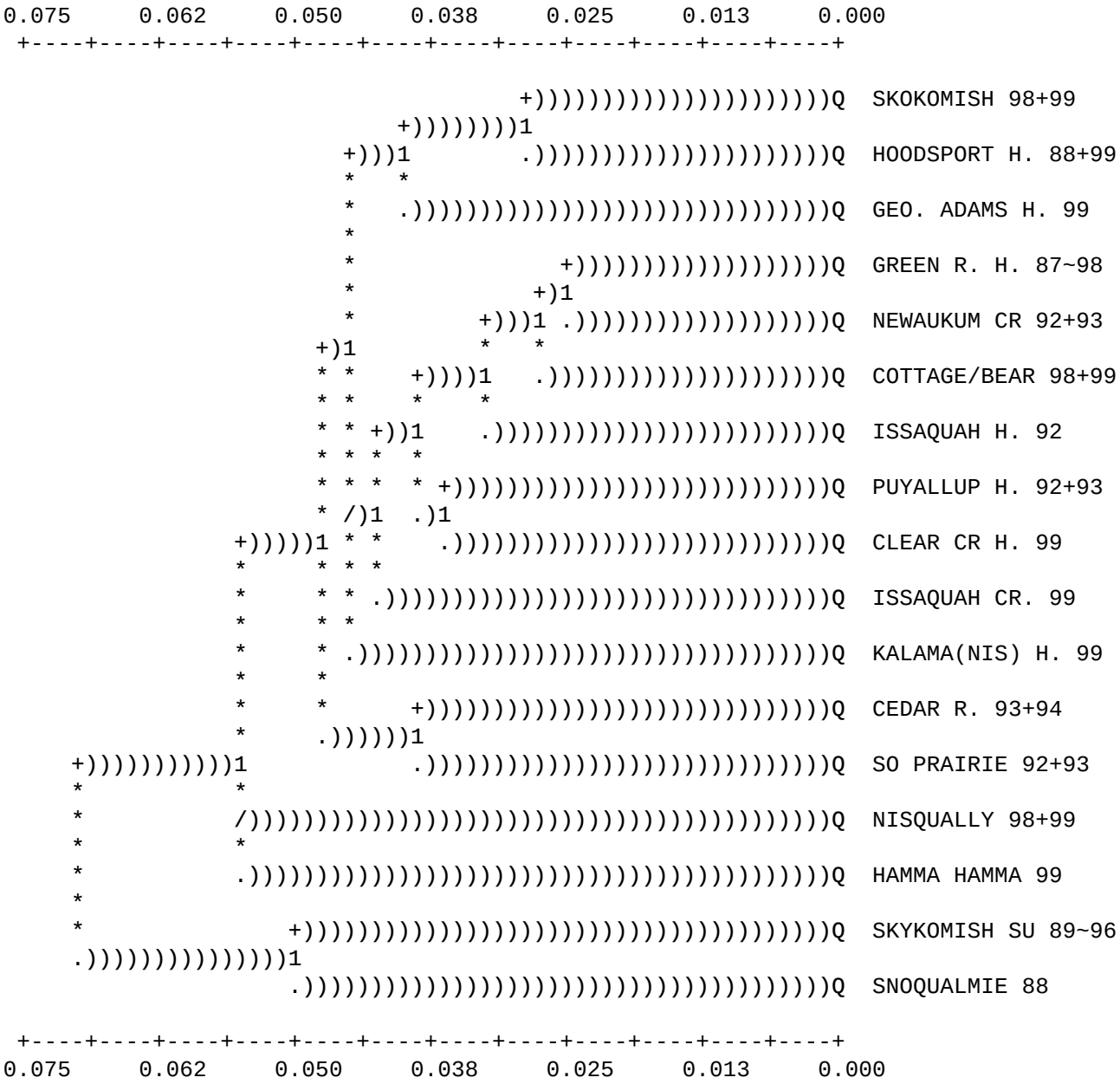

Figure 4. Multidimensional scaling diagram of genetic distances among combined Cottage Lake/Bear creeks sample, other Lake Washington area, and south Puget Sound samples.